THE EFFECT OF ROENTGEN RAYS ON THE COLLOIDAL PROPERTIES OF THE STARFISH EGG

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In order to determine how protoplasm is affected by irradiation, ordinary methods of microscopic examination are insufficient. For marked changes in the protoplasmic colloid, changes which might even involve death of a cell, are usually not reflected in the microscopic appearance of dead, fixed, or even of unfixed cells. Similarly, cytochemical methods usually fail to give evidence of alterations in the colloidal organization of the cell. In order to understand how roentgen rays affect living matter one must take into account the fact that protoplasm is a living colloid and determine how irradiation affects this colloid.

Wilson (1950) discovered viscosity changes in the irradiated *Arbacia* egg during mitosis. He was, however, unable to detect such effects in the unfertilized *Arbacia* and *Chaetopterus* egg. These negative results were confirmed (Rieser, unpublished data) even with much higher irradiation doses (200,000 r) than those used by Wilson. One might suppose that colloidal changes nevertheless do occur in the unfertilized egg following irradiation but that they do not become manifest until the egg is in its mitotic cycle or is physiologically active in some other way. This view is in agreement with the common belief that living matter is more highly radio-sensitive while undergoing some physiological change than when it is relatively inactive. With these views in mind experiments were performed, and are here reported, to elucidate the effects of roentgen rays on the unfertilized starfish egg.

Materials and Methods

Eggs from the starfish *Asterias forbesii* were obtained by removing the ovaries from ripe females and placing them in finger bowls containing sea water. The loose eggs were strained through a double layer of cheesecloth in order to remove all debris and placed in sea water-containing stender dishes in a constant-temperature water bath at 23° C.

Egg suspensions were irradiated in plastic dishes immediately after obtaining the eggs. The characteristics of radiation were 180 k.v.; 25 m.a.; 6000 roentgens per minute; target distance 9.5 cm.; equivalent inherent filtration equal to 0.2 mm. of copper. Two opposed, parallel, self-rectifying tubes were used simultaneously.

Protoplasmic viscosity determinations were made with the centrifuge method. Only those eggs whose germinal vesicles had broken down were used. Eggs in

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sea water were placed in glass centrifuge tubes containing a pycnotic cushion of 0.73 *M* sucrose which was separated from the egg suspension by an air space. An Emerson electric centrifuge was operated at top speed (14,200 r.p.m.), developing a force of 22,600 times gravity at this speed. In each experiment eggs were subjected to this centrifugal force for increasing periods of time, from 5 seconds upward. At each centrifugation time a different aliquot of the same egg suspension was tested. In each aliquot 50 eggs were examined and the percentage of cells with hyaline zones was noted. In some of the experiments the number of eggs with fat caps was determined. A study was also made to determine if roentgen radiation alters the colloidal properties of the nucleoplasm. Eggs in the germinal vesicle stage were used. Measurements of nuclear viscosity were made with the falling nucleolus method (Harding, 1949). Eggs were arranged on the stage of a horizontal microscope, and the time required for the nucleolus to fall through the entire diameter of the nucleus was measured. This was usually repeated ten times with each egg. The author wishes to thank Dr. Clifford V. Harding for suggesting the experiments on the nucleus and for his advice and criticism of this part of the work.

Results

The results of the effect of roentgen rays on the relative protoplasmic viscosity and on the appearance of fat caps in unfertilized starfish eggs are presented in Tables I and II. Six experiments were performed using 50,000 r (Table I) and five experiments with 100,000 r (Table II). In general a far greater percentage of unirradiated eggs possess hyaline zones after centrifugation than do the irradiated eggs. This holds true over the whole range of centrifugation times employed and is particularly noticeable below 60 seconds, with both irradiation doses (50,000 r, Table I and 100,000 r, Table II). Thus, x-rays markedly increase the protoplasmic viscosity of the unfertilized starfish egg. The effect, however, is no more pronounced with the higher dose of x-rays. Both tables also show that

Table I

The effect of roentgen rays (50,000 r) on protoplasmic viscosity and fat release in the starfish egg

Time of centrifugation, sec.	Controls, aver.% hyaline zones	Irradiated, aver. % hyaline zones	Controls, aver. % fat caps	Irradiated, aver. % fat caps
5	2	0		
10	1	0		
15	2	0		
20	3	0		
25	4	1		
30	2	1		
35	10	0		
60	17	13		
90	28	15	40	60
120	17	15	51	17
150	31	12	41	49
180	36	18	24	66

TABLE II

The effect of roentgen rays (100,000 r) on protoplasmic viscosity and fat release in the starfish egg

Time of centrifugation, sec.	Controls, aver. % hyaline zones	Irradiated, aver. % hyaline zones	Controls, aver. % fat caps	Irraidated, aver. % fat caps
5	3	0	15	15
10	7	1	16	14
15	9	1		
20	12	3		
25	12	1		
30	16	6	7	14
45	36	32	12	34
60	50	30	27	56
90	18	6	12	22
120	32	4	20	38

there is a somewhat greater number of eggs showing fat caps after centrifugation in the irradiated eggs than in the non-irradiated controls. More striking is the relatively far greater proportion of irradiated eggs with fat caps despite the higher protoplasmic viscosity of the latter (and consequently a decreased tendency toward centrifugal displacement of intracellular components in these eggs). The ability of x-rays to increase the percentage of eggs with fat caps therefore demonstrates an intracellular release of lipid materials. In addition to viscosity changes and fat release two further effects of irradiation were noted following centrifuga-

TABLE III

The effect of roentgen rays on time of fall of nucleolus and the thixotropy of the nuclear colloid

Dose, r	Time for initial fall, sec.	Aver. of times for subsequent falls, sec.	Ratio initial time to aver. of subsequent times
0	187	171	1.094
	210	187	1.123
	200	168	1.190
	210	181	1.160
	211	167	1.263
	212	178	1.191
	152	150	1.013
	150	145	1.035
	174	162	1.080
	184	178	1.033
50,000	160	111	1.441
	157	133	1.180
	166	142	1.169
100,000	240	148	1.623
	216	167	1.293
	170	185	0.919
	149	131	1.137
	210	132	1.599
200,000	160	144	1.111
	187	160	1.169

tion. In unirradiated eggs exposed to prolonged centrifugation there is always a number of cells that become destroyed. In such cells, the membrane separates from the cytoplasm, giving the appearance of a so-called "ghost" membrane similar to that of the mammalian erythrocyte. Irradiation tends to prevent this "ghost" formation. Thus, for example, using 50,000 r and centrifuging for 150 seconds, 8% of the unirradiated controls were ghosts, as compared with 0% ghosts in the irradiated eggs. Irradiation was found also to prolong the centrifugation time required to produce shape changes (*i.e.*, elongation) of the eggs. Table III contains the data pertaining to the effects of x-rays on the nuclear colloid. In the second column, the time of initial fall of the nucleolus through the germinal vesicle is a measure of viscosity (since the temperature was constant, viscosity is proportional to time). Since there was too much variability of the time of fall of the nucleolus among individual eggs, a comparison of the nuclear viscosity in the control and irradiated eggs cannot be made. The last column in Table III gives the ratio of the initial time of fall of the nucleolus to the average of subsequent falls and is therefore an index of thixotropy of the nuclear colloid. Application of the t-test to the means of these ratios reveals that there exists no significant difference in nuclear thixotropy between the control and irradiated eggs.

Discussion

A fundamental question arises through the finding that x-rays alter the protoplasmic viscosity of the unfertilized *Asterias* egg but not that of the unfertilized *Arbacia* or *Chaetopterus* egg. How may one account for this difference in colloidal behavior? The answer may be found if one examines the modes of maturation in each case. The stage of maturation, and particularly the time course in which it is attained in the starfish egg, as compared with the other two cases, appears to be of decisive importance in determining the radiosensitivity of the protoplasmic colloid. The *Arbacia* egg, when shed into sea water, is fully matured, both polar bodies having been formed within the ovary. In the *Chaetopterus* egg, maturation begins upon its removal from the ovary into sea water, progresses to the metaphase of the first maturation division and ceases. This stage is reached within 15 minutes after the eggs are placed into sea water. In the *Asterias* egg, however, according to Tennent and Hogue (1906), the first maturation division is not completed until 70 minutes, and the second division not until after 105 minutes, after its removal from the ovary. Since the procedures of washing the eggs, preparing them for irradiation, and the irradiation itself generally took at least an hour, usually more, the starfish eggs actually were in the process of maturation during the x-ray treatment, while the eggs of the other two species were not. Thus, during the time the x-rays acted on the starfish egg, the latter was undergoing a physiological process, that of maturation. This is in accordance with the belief that protoplasm is more highly radiosensitive when in a state of activity. As in the *Arbacia* egg during mitotic division where irradiation prolongs the mitotic gelation, so does the starfish egg show an increased protoplasmic viscosity following its maturation divisions. The direction of the viscosity change in the irradiated starfish egg, that of increase, is the same as that observed by Wilson (1950) in the irradiated *Arbacia* egg during mitosis. In the latter case, the viscosity of the treated egg remains high for a period of about two

or three times that of the controls. Wilson postulates that the effect of irradiation is an alteration or destruction of heparin within the egg, thus favoring prolonged gelation. He cites literature in support of this belief. His hypothesis might also be advanced in the case of the unfertilized, irradiated starfish egg.

The increase, especially seen as a relative increase, of fat caps following irradiation, is not surprising in view of previous studies where irradiation is known to increase the number and size of fat droplets in cells (Nadson and Stern, 1931), and in view also of the lipemia in mammalian blood following total-body irradiation. The effect may be due to a breakdown of protoplasmic protein-lipid complexes into proteins and lipids.

The tendency of irradiated starfish eggs to resist centrifugation-induced shape changes and membrane separation suggests that x-rays increase the cortical, or surface, rigidity of the eggs as they increase the viscosity of the interior protoplasm.

Summary

1. Unfertilized eggs of the starfish, *Asterias forbesii,* were irradiated with single x-ray doses of 50,000 r and 100,000 r, respectively.

2. Roentgen irradiation produces a marked increase in protoplasmic viscosity. An explanation is given for the absence of colloidal changes following irradiation of eggs of two other species.

3. Two further radiation effects are: intracellular release of fat, and increase in cortical, or surface, rigidity of the starfish eggs.

4. Doses as high as 200,000 r fail to have a detectable influence on the nuclear colloid of the starfish egg.

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